REMARKS

All claims have been set forth with appropriate status identifiers. In this connection, the printer which made the undersigned's file copy of the last amendment substituted asterisis where a bond line should appear in claims 9 and 26. Since it is believed that the amendment submitted correctly set forth bond lines, this has been corrected without any indication of amendment.

Claim 17 has been amended to indicate that the lipids, cholesterol and surfactants are liposome-forming agents in light of the Examiner's observation. While it is believed that the language in the claims was clear that the material forming the liposomes included at least one cationically charged component present in an amount such that the vesicles have an overall cationic charge, some clarifying language has been added to claim 17. As to the intravascular space, the word "the" has been changed to "an". In light of the foregoing amendments, it is respectfully submitted that the rejection based on 35 U.S.C. § 112 can now be withdrawn.

Claims 1, 2, 6, 8-14, 16-20, 25-31 and 34-38 were rejected under 36 U.S.C. 103 over Felgner in view of Kirby and Weiner. This rejection is respectfully traversed.

A review of Felgner will show that the reference lacks any teaching or suggestion of cationic liposomes in which polynucleotides encoding immunogens are entrapped in the intravascular space. There is no teaching in this reference of any *in vivo* results. All transfection experiments described were carried out on cell lines *in vitro*.

Felgner thus teaches cationic lipids and their use in various applications, one of which is for transfecting polynucleotides into cells. There is no teaching or suggestion about a method of generating a cell-based and humoral immune response to

a target polypeptide. There is no suggestion that a cell-based and humoral immune response is even a remote possibly.

The reference to the use of lipids is very broad and covers a variety of liposomal and non-liposomal systems. At column 7, lines 49-51, there is a reference to a polynucleotide coding for an immunogen but without a reference to any particular delivery system. Later in this disclosure, particularly at column 8, line 21 et seq., there is a reference to preparing lipid vesicles and using the lipid vesicles to facilitate the transfectional transport of bioactive agents into cells but there is no reference in this material to DNA encoding an immunogen nor is there any reference in that paragraph to the way in which the lipid vesicles and the active can be associated with one another.

Still later in Felgner's description, namely at column 15, line 7 et seq., there is a description of forming liposomes in which a film of lipid is formed on a surface and then hydrated to form the liposomes. A bioactive agent is captured within the liposomes either by being present in the aqueous suspending solvent or by being mixed with lipid before forming the dry film. There is a reference in this paragraph also to example 12 where empty liposomes are formed by that general method.

The only reference formulating polynucleotide in the working examples can be found in Examples 13 and 15-20. In Example 13, the polynucleotide is associated with the cationic lipid by forming a complex but there is no step in the complex forming process which results in entrapment of polynucleotide into the intravascular space of the liposomes. Example 15 does not even describe how the lipids and polynucleotide is mixed and presumably, therefore, it is by the same process as in Example 13. This polynucleotide does not encode an immunogen.

To overcome the deficiencies in Felgner, the Office Action relies on Kirby but this additional art is silent as to DNA encoded with an immunologic polypeptide. DNA vaccines had not been invented as of the date of the publication of Kirby and that means that polynucleotide encoding an immunologic polypeptide in a function manner was not a part of the prior art as the publication date of Kirby in 1984 and could not have been contemplated by the authors. Furthermore, the document which Kirby cross references as disclosing further information about the DNA used in the entrapment of table 1, namely reference number 9, indicates that the DNA is E. coli DNA and no other characteristics of the DNA is given. Kirby also fails to show that any activity of the entrapped DNA is retained. Further, no reference is made to the delivery of the DNA, much less that the DNA would have activity after administration *in vivo*. The best argument that can be made is that it would be obvious to try the Kirby process with Felgner and see what happens but "obvious to try" does not satisfy the requirements of Section 103.

The assertion on page 6 of the Office Action that the skilled person would have been motivated to use the Kirby dehydration/hydration method to encapsulate DNA because it provides excellent encapsulation yields is respectfully submitted to be a hindsight justification which is not supported by either reference or elsewhere in the prior art. There is no teaching in either reference about generating a cell-based and humoral immune response and there is nothing in either reference to motivate a skilled person to try achieve such an effect. Kirby is silent about immune responses and there is nothing in Felgner about any particular type of immunity which could be generated upon deliver of the components. The discussion in column 18 does not include how one might measure any effect on the immune system, still less that the effect is cell-based or humoral, and even still less, that it should be both types of immune responses.

The combination of these references is not proper. Felgner is limited to cationic lipids which are clearly electrostatically opposite to the nucleic acids which are examples of the active materials which may be entrapped. The liposomes formed of cationic lipids in Felgner are used to form complexes with nucleic acids in which the nucleic acid is ionically bound to a preformed liposome. In that connection, the examples in the present application show that such complexes with the nucleic acid on the outside of the bi-layer (that is, outside the liposome) has very different properties compared to the liposomes of the present invention in which nucleic acid is in the intravascular space. In contrast to Felgner, the Kirby interaction between the liposomes and their nucleic acid is not ionic and the nucleic acid is entrapped into the intravascular space of liposomes having no overall charge. In other words, the interaction between the liposome forming components and the active material in the two references is totally different. Clearly, there would be no motivation to combine these two references when faced with the desire of providing a method for generating cell-based and humoral immunity. The Kirby method provides an arrangement of liposomes and actives inside the intravascular space of the liposomes while Felgner provides an interaction on the exterior surface by electrostatic extraction. There is clearly no reason to have an expectation of success.

Tables 3 to 5 in Example 2 show significant differences in immune response between a Felgner type process (Example 2.4) and the entrapment products of the present invention (Example 2.3). For the same lipid composition (PC:DOPE:DOTAP, 1:0.5:0.25), there is a significant difference in immune response where the higher level of DNA is entrapped, at both 28 and 21 days. Example 3 shows cytokine levels are significantly different and that there is both humoral and cell-mediated immunity. These differences are also apparent in Example 4 which show humoral immunity differences. In Example 10, further experiments showing the differences between the

products of the complexing and entrapment processes and clearly show complexation produces a product having a different conformation. See also, the results and discussions on page 41, line 7 to page 45, line 2 and page 46, line 6 to page 51, line 22. The remaining examples show that entrapped nucleic acid, when administered, does provide the humoral and cell mediated immune effect.

The Office Action further asserts that the Kirby method would exclude nucleases with greater success using a oligolamellar and multilamellar vesicles rather than unilamellar vesicles based on page 982 of that reference. However, this description relates to the loss of a diffusible entrapped solute from the inside of the vesicle. It appears that the contention is that the nucleases would be excluded more successfully but if this understanding is correct, it is respectfully submitted any basis for the assertion on the loss of solutes does not appear to have any support in fact or logic. There is no disclosure that nucleases attack nucleic acids, still less that it takes place by nucleases diffusing into the liposomes and even further less that the rate of diffusion of a nucleases into an oligolamellar liposome would be reduced as compared to a unilamellar liposome. Nucleases are very different from carboxyfluorescein, the solute being discussed by Kirby, which has susceptible to diffusion out of the liposomes. Nucleases are proteins with enzymatic activities having high molecular weights.

In light of the foregoing considerations, it is respectfully submitted that the combination of Felgner and Kirby is inappropriate and would not be done by one skilled in the art, except by using hindsight to meet the explicit requirements of the instant claims and that, of course, is improper.

Weiner is relied upon solely to teach methods of causing an immune response to an individual by injection of a polynucleotide encoding an immunogen. It

is not asserted to, nor does it cure, the basic deficiencies in the combination of Felgner and Kirby.

Claim 7 was rejected under 35 U.S.C. 103 in view of Felgner, Kirby, Weiner, and Collins. This addition reference, Collins, has been relied on solely to show microfluidisation and for a method of making dehydration-rehydration cationic liposomes. It does not, however, suggest any particular lipids or classes of lipids are useful with any specific actives or classes of actives and contains no teaching or suggestion that the nucleic acids described encode an immunogen. It does not describe a method which involves hydration in the presence of an active but instead describes the situation where the active is mixed with a dried lipid powder, as indicated at column 5, lines 3-9. Thus, the additional reliance on Collins does not cure any of the deficiencies in the other combination of references.

Claims 1, 3, 6, 8-14, 16-20, 22, 25-31, 34, 35, 37 and 38 were rejected under 35 U.S.C. 103 over Felgner in view of Kirby and Liu. This rejection is also respectfully traversed.

The combination of Felgner and Kirby has been discussed above. Liu has been cited for teachings relating to polynucleotides. It is asserted that the skilled person would have been motivated to use polynucleotides in Felgner as modified by Kirby because "both Felgner and Weiner (sic; Kirby???)" suggest that liposomal compositions should be used for *in vivo* delivery of nucleic acids encoding immunogens. There is, as discussed above, no proper basis for combining Felgner and Kirby. Felgner has no disclosure suggesting liposomal compositions should be used for *in vivo* delivery of nucleic acids encoding immunogens but instead describes "direct injection of cationic lipids together with DNA, RNA...into cells of an animal *in vivo*" at column, line 33 but there is no disclosure of the method in application claim 1, namely

subcutaneous or intramuscular injection. Rather the procedures disclosed must involve direct injection into cells. The combination of Liu and Kirby does not result in the invention claimed in this case.

Finally, it is noted that in the response to arguments, there are two replies which are based on the statement that there is "no reason" to assume that what is disclosed by a reference does not meet the claims. While grammatically a double negative is a positive, both of these "no reason to assume" contentions are constructs based on a lack of expressed negative statement, i.e., the statements are based on silence. It is well established that it is improper to rely on silence.

In view of the above amendment, applicant believes the pending application is in condition for allowance.

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Respectfully submitted,

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